Supplementary Information

Enzyme/quantum dots architecture for highly sensitive electrochemiluminescence biosensing of oxidase substrates

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Reagents. Glucose oxidase (GOD, E.C. 1. 1. 3. 4, from *Aspergillus niger*, 39800 U mg^{-1}) and β-D(+)-glucose were purchased from Sigma. Other reagents were of analytical reagent grade. All solutions were prepared with doubly distilled water. The paraffin impregnated graphite electrode (PIGE, 3.0 mm radius) was prepared and pretreated according to the reference (*Anal. Chem.*, 2004, **76**, 6871-6876.)

Apparatus. Electrochemiluminescence (ECL) was conducted with a homemade ECL system comprising a QDs or GOD/QDs architecture modified working electrode, a Pt counter electrode, and a Ag/AgCl reference electrode. During measurements, the cathodic potential was applied to the working electrode by cyclic voltammetric (CV) technique via a CHI 812 electrochemical working station (Co. CHI, USA). The ECL emission was detected synchronously with a luminescence analyzer (IFFM-D, Remax Electronic Instrument Limited Co., Xi'an, China) at room temperature. Electrochemical impedance spectroscopy (EIS) was performed with CHI 660 electrochemical analyzer (Co. CHI, U.S.A) in 10.0 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) containing 10.0 mM KCl. UV-vis absorption spectra were acquired with a Perkin-Elmer lambda 35 photospectrometer (Perkin Elmer Co., USA). FT-IR spectra were recorded on a

Vector 22 FT-IR spectrometer (Brucker Co., Germany). Photoluminescence was performed on a RF5301 fluorophotometer (Shimadzu, Japan). Scanning electron micrographs (SEM) of different films were obtained with a LEO 1530 VP scanning electron microscope (Leo Ltd., Germany), equipped with an Inca 300 EDS (Oxford Ltd., England).

Preparation of TGA-Capped CdSe QDs. TGA-capped CdSe QDs were synthesized by the following procedure: 20 mL 5 mM $CdCl_2$ was mixed with 20 μL of TGA, and 1 M NaOH was added to adjust pH to 11.2. The clear solution was diluted to 50 mL and bubbled with high-purity N_2 for 30 min. Then 0.5 mL of 0.1 M Na_2SeSO_3 was injected to this mixture to obtain a light yellow clear solution of TGA-capped CdSe QDs. The final molar ratio of Cd^{2+}:TGA:Se^{2-} was about 1:2.5:0.5. The sizes of the obtained QDs could be tuned by simply varying the reflux time. The QDs solution was then dialyzed against water for 48 hours and centrifuged to remove a morsel of precipitate. The final solution could be rather stable for 2 months when kept in a refrigerator at 4 ^{o}C.

Preparation of QDs Film and GOD/QDs Architecture on PIGE. The QDs films were formed by casting 15 μL obtained QDs aqueous solutions on PIGEs. The GOD/QDs architectures were fabricated by the consecutive cast with a series of volumes of 1 mg/mL GOD and 0.021 mM QDs (2.5 nm in size) solutions on the PIGEs. After dried in air at 4 °C the formed GOD/QDs architectures were washed with double-distilled water and stored in 0.1 M pH 7.0 PBS at 4 °C. As compared, QDs-GOD bioconjugate was prepared as the classical method (G. T. Hermanson, Bioconjugate Techniques, Academic Press, New York, 1996) and the film of the bioconjugate was fabricated with the same steps.

Characterization of synthesized QDs. The UV-vis spectra (Figure S2, left) of a series of TGA-capped CdSe QDs are obtained by varying the reflux time to tune the sizes. According to the following empirical equations, the sizes and concentrations of QDs can be estimated from the adsorption peaks in UV-vis spectra. The results are shown in Table S1.

$$D\ (\text{nm}) = (1.6122 \times 10^{-9})\lambda^4 - (2.6575 \times 10^{-6})\lambda^3 + (1.6242 \times 10^{-3})\lambda^2 - (0.4277)\lambda + (41.57)$$

$$C\ (\text{mol L}^{-1}) = A/[5857\ (D)^{2.65} l]$$

The unit of parameters λ and l is nm and cm, respectively.

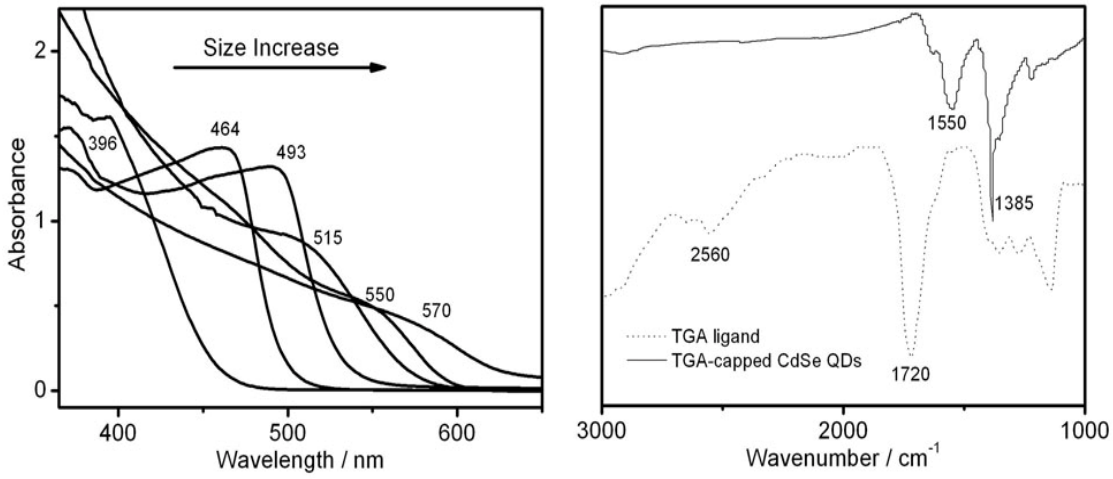

Figure S1. (left) Uv-vis spectra of a series of QDs obtained after dialysis and 1:1 dilution with water. (right) FT-IR spectra of TGA and TGA-capped CdSe QDs.

The FT-IR spectrum (Figure S1, right) of 2.5 nm TGA-capped CdSe QDs shows the diminishment at 2560 cm^{-1} peak for stretch vibration of S-H bond in TGA molecule, indicating the formation of S-Cd bonds between TGA and CdSe core. The shift of the asymmetric vibration of carboxyl group in TGA from 1720 to 1550 cm^{-1} implies that the –COOH in TGA turns to its anion, which also leads to the appearance of the symmetric vibration of carboxyl anion at 1385 cm^{-1}. In a whole, the structure of

the obtained QDs can be identified as excess TGA molecules cover cadmium-riched CdSe core.

Size effect of QDs. The sizes of TGA-capped CdSe QDs affect the ECL intensity and peak potential, as shown in Table S1. The selected value in this work is 2.5 nm in size.

Table S1. Characterization and size effect of QDs

λ (nm)	d (nm)	Concentration (mM)	ECL intensity (Normalized)	ECL peak potential (V vs Ag/AgCl)
396	1.5	0.182	0.17	-1.59
464	2.0	0.072	3.86	-1.51
493	2.3	0.050	12.7	-1.40
515	2.5	0.021	100	-1.10
550	3.0	0.009	25.8	-1.03
570	3.5	0.005	2.73	-1.03

EIS measurements. The EIS plots of the redox probe $[Fe(CN)_6]^{3-/4-}$ at bare and GOD/QDs architecture modified electrodes are shown in Figure S2. The formed GOD/QDs architecture showed a charge transfer resistance (R_{ct}) value of only 0.3 kΩ, which was much lower than that of about 7.4 kΩ for the QDs-GOD bioconjugate film.

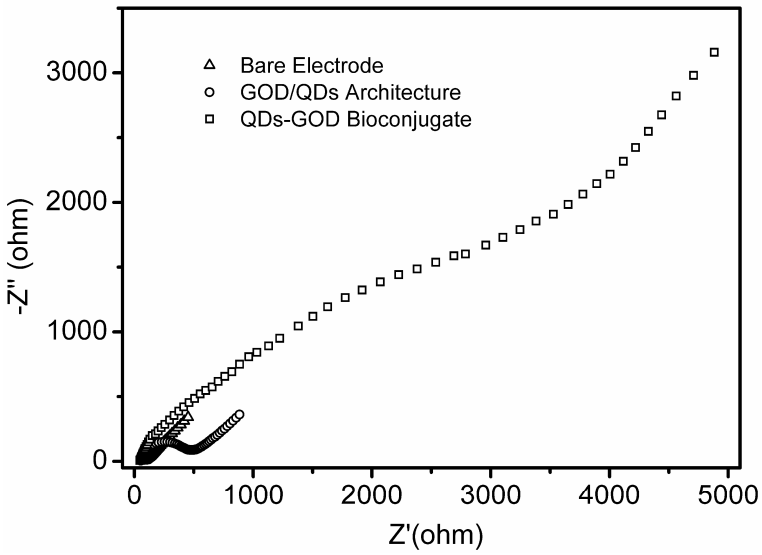

Figure S2. EIS plots for bare, GOD/QDs architecture and QDs-GOD bioconjugate modified electrode in the solution of 10 mM $[Fe(CN)_6]^{3-/4-}$ containing 10 mM KCl.

Optimization of the detection. Figure S3 shows the effects of different parameters for preparation of the GOD/QDs architecture and the glucose detection on the ECL signal. The optimal parameters are 5.0 μg GOD, 0.3 nmol QDs, pH 7.0 PBS and a scan rate of 400 mV s^{-1}, which are adopted in this work.

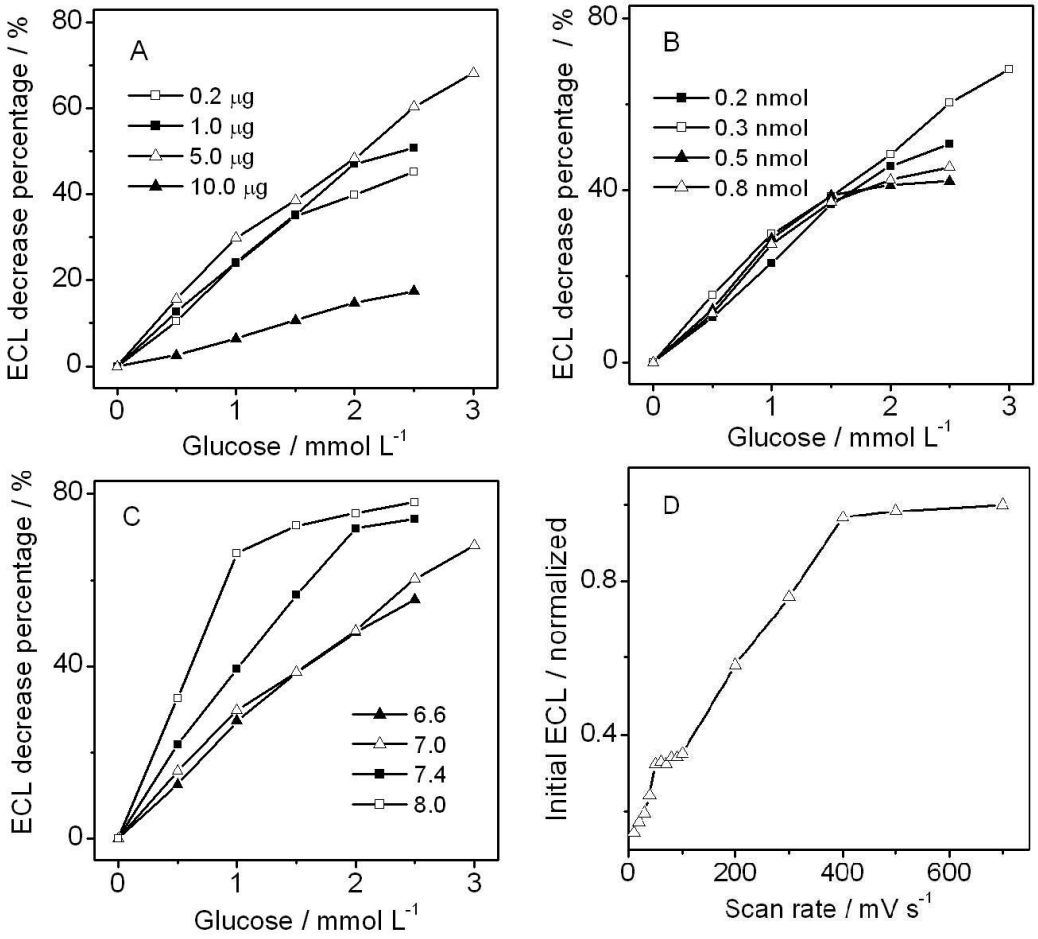

Figure S3. Optimization of (A) the amount of GOD; (B) the amount of QDs; (C) buffer pH and (D) scan rate. The normalized ECL intensity (identified as 1.0) in figure D is corresponding to the ECL of the architecture at 400 mV s^{-1} in 0.1 M pH 7.0 glucose-free PBS. All other factors are at their optimal values during optimizing one parameter.

Reproducibility of the strategy. The results of intra-assay and inter-assay deviations are shown in Figure S4.

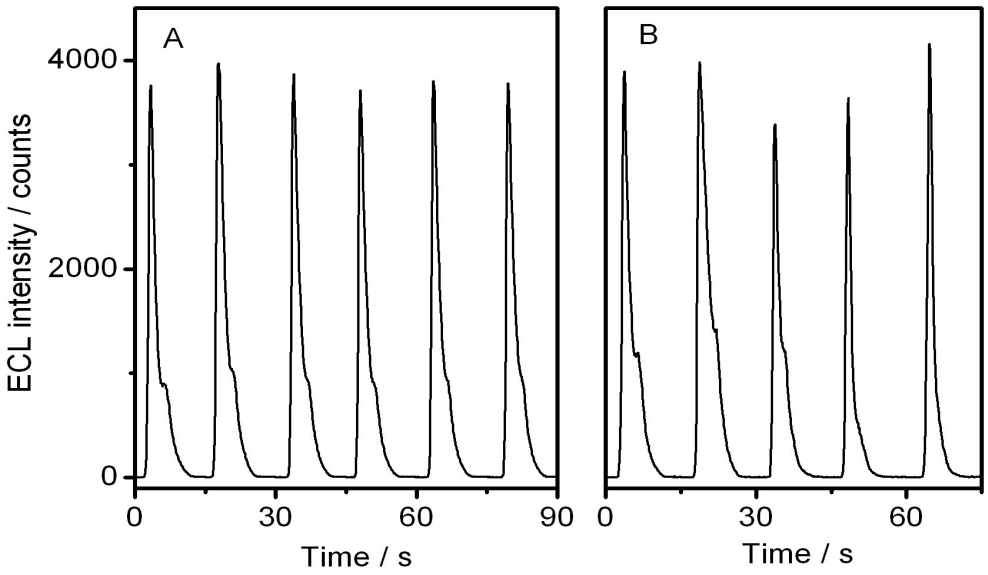

Figure S4. (A) six parallel measurements with one optimized electrode and (B) measurements with five electrodes fabricated independently upon injections of 2.0 mM glucose into the detection solution.

Interference. In the interference study, the tolerable concentration ratio for a 5% signal change is assessed. Common inorganic ions such as Na^+, K^+, Cl^- and SO_4^{2-} at 500 times concentration of glucose; EDTA, oxalate and citrate at 100 times concentration of glucose show little influence. Only equimolar ascorbic acid and uric acid may interfere with the detection since they may quench some intermediates generated in the cathodic scan and induce the decrease in ECL. The interference can usually be eliminated with addition of ascorbate/urate oxidase.

Sample detection. The glucose in serum sample is diluted with the optimized buffer solution in a series of ratios. At each ratio three uniform detections are carried out. The mean values of 11.70 (for 50 times dilution), 11.18 (100 times dilution) and 10.90 mmol L^{-1} (133 times dilution) are obtained. Compared with the reference value of 10.68 mmol L^{-1}, the relative errors are 9.5, 4.7 and 2.1%, respectively, indicating acceptable accuracy.